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REGULAR ARTICLE

Tryptophan versus nitric oxide, nitrogen dioxide and carbonate radicals: differences in reactivity and implications for oxidative damage to proteins

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Abstract The reactions of carbonate ($CO_3^{\cdot-}$), nitric oxide ($NO^{\cdot}$) and nitrogen dioxide ($NO_2^{\cdot}$) radicals with free zwitterionic tryptophan and *N*-formyl-tryptophanamide (a model for tryptophan as a protein residue) have been studied using density functional theory and transition state theory. All possible reactions mechanisms have been analyzed. They are single electron transfer (SET), radical adduct formation and formal hydrogen transfer. The aqueous solution has been mimicked at physiological pH. Thermochemical and kinetic data are reported for both tryptophan models. We find that the reaction rate constants for $CO_3^{\cdot-}$ with both tryptophan models are limited by diffusion, while for reaction with $NO_2^{\cdot}$ they are approximately 3.00×10^6 M^{-1} s^{-1}, and $NO^{\cdot}$ does not react at all. The overall rate constants of free zwitterionic tryptophan with $NO_2^{\cdot}$ and $CO_3^{\cdot-}$ are 1.11 and 1.29 times larger than those of the *N*-formyl-tryptophanamide model, respectively. Therefore, it seems that the free amino acid and the residue in the protein have similar reactivities. While $CO_3^{\cdot-}$ reacts via all three studied mechanisms at similar rates, $NO_2^{\cdot}$ reacts exclusively via SET. Our work suggests that free tryptophan has some scavenging activity and protective effect, but that bonded tryptophan could be a target for oxidative stress.

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Keywords Rate constants · Reaction mechanisms · Alpha amino acid tryptophan · Oxidative stress · Protein damage

1 Introduction

Nitric oxide ($NO^{\cdot}$) is a free radical whose production is ubiquitous in the human body. It has been suggested that $NO^{\cdot}$ is a highly reactive radical [1] capable of damaging even some of the less reactive amino acids, such as leucine, isoleucine and valine [2]. It has also been proposed that $NO^{\cdot}$ reacts with the tyrosyl and tryptophanyl radicals in peptides and proteins at diffusion-controlled rates [3]. However, the related experiments are conducted in conditions that do not necessarily prevent reactions of $NO^{\cdot}$ with molecular oxygen (O_2) or superoxide anion radical ($O_2^{\cdot-}$). In these reactions, nitrogen dioxide radical ($NO_2^{\cdot}$) and peroxynitrite ($ONOO^-$) are formed, respectively, and these are known to be reactive nitrogen species (RNS). In other words, $NO^{\cdot}$ is an acknowledged source of other RNS which are responsible for a wide array of diseases, including Alzheimer's disease, atherosclerosis and stroke [4]. In these settings, $NO^{\cdot}$ and $O_2^{\cdot-}$ can combine in a near diffusion-rate limited reaction to form $ONOO^-$ [5]. In turn, $ONOO^-$ has the ability to modify a variety of amino acids in proteins, including oxidation of sulfur-containing amino acids (cysteine and methionine) and nitration of aromatic amino acids (tyrosine, tryptophan, phenylalanine and histidine), often resulting in modulation of the modified protein's function [6]. $ONOO^-$ formation is maximal when equal amounts of $NO^{\cdot}$ and $O_2^{\cdot-}$ are present. $ONOO^-$ may either directly react with amino acids or it could be a precursor of other reactive radicals, $NO_2^{\cdot}$ and hydroxyl radical ($^{\cdot}OH$). Additionally, $NO^{\cdot}$ can react with O_2 to form $NO_2^{\cdot}$, with a rate constant of 2.30×10^6 M^{-1} s^{-1} at

Table 1 Kinetic experimental data

Reaction	k (M^{-1} s^{-1})	pH	References
$NO^{\cdot} + O_2 \rightarrow NO_2^{\cdot}$	2.30E+06	7.0	[7]
N-ac-TrpH-NH_2 + $NO_2^{\cdot}$	1.10E+06	7.3	[19]
Tryptophan + $NO_2^{\cdot}$	1.00E+06	6.5	[9, 21]
Tryptophan + $CO_3^{\cdot -}$	1.00E+08	10.0	[20]
Tryptophan + $CO_3^{\cdot -}$	7.00E+08	7.0	[9, 22]
Tryptophan + $CO_3^{\cdot -}$	4.40E+08	11.2	[23]

pH 7.0 in water solution [7] (Table 1). This reaction can only be avoided in a strictly inert atmosphere, which is certainly not the case in biological media. $ONOO^-$ reacts with carbon dioxide (CO_2) to form the reactive adduct nitrosoperoxycarbonate ($ONOOCO_2^-$), which decomposes to carbonate radical ($CO_3^{\cdot -}$) and $NO_2^{\cdot}$ [8]. $CO_3^{\cdot -}$ and $NO_2^{\cdot}$ could be involved in dityrosine formation and cross-linking between tryptophanyl radicals, two reactions that have been associated with pathophysiological processes [9–12]. Therefore, it is important to compare the reactivity of $NO^{\cdot}$, $CO_3^{\cdot -}$ and $NO_2^{\cdot}$, since they are capable of damaging some amino acids and their formation is closely related under physiological conditions.

$NO_2^{\cdot}$ has been proposed to react with various compounds via addition to double bonds [13, 14], hydrogen abstraction [15] or electron transfer mechanisms [16]. It has been reported that this radical oxidizes tyrosine, tryptophan, cysteine, several proteins, and the anions of unsaturated fatty acids linoleic and arachidonic acid [17, 18]. Domazou et al. [19] showed that the reaction of $NO_2^{\cdot}$ with *N*-acetyl-L-tryptophanamide (*N*-ac-TrpH-NH_2) was followed by the formation of the corresponding amino acid radicals, as monitored by the transient absorbance changes at 510 nm, ε_{510}(*N*-ac-Trp$^{\cdot}$-NH_2) = 2000 M^{-1} cm^{-1} with the following rate constant.

$$N\text{-ac-TrpH-NH}_2 + NO_2^{\cdot} \rightarrow N\text{-ac-Trp}^{\cdot}\text{-NH}_2 + NO_2^- + H^+ k = 1.1 \pm 0.1 \times 10^6 M^{-1} s^{-1}$$

They also found that the rate constant of the reaction of $NO_2^{\cdot}$ with iron(II)cytochrome c (tryptophan containing protein) is about 15 times slower than that of $CO_3^{\cdot -}$ [20]. Independently, Ohara et al. [9] and Walter et al. [21] showed that the rate constant of the reaction between $NO_2^{\cdot}$ and tryptophan is ~1.00×10^6 M^{-1} s^{-1} (Table 1).

In the case of the rate constants for the reactions of $CO_3^{\cdot -}$ radicals in neutral aqueous solution with aromatic amino acids, they are expected to be larger than 10^8 M^{-1} s^{-1}, particularly for amino acids with indole group and its derivatives [22]. Schoen-nan et al. [22] and Ohara et al. [9] found that the rate constant for the reaction of the carbonate radical with tryptophan is equal to 7.00×10^8 (pH = 7.0) M^{-1} s^{-1}. Domazou and Koppenol [20] proposed that reaction between $CO_3^{\cdot -}$ and tryptophan is ~1.00×10^8 (pH = 10.0) M^{-1} s^{-1}, while Adamsg et al. [23] found it to be equal to 4.40×10^8 (pH = 11.2) M^{-1} s^{-1}, and all these rate constants are shown in Table 1. In this work, we shall elucidate the reaction mechanism of tryptophan toward this radical by performing a kinetic study, considering physiological conditions, i.e., pH = 7.4.

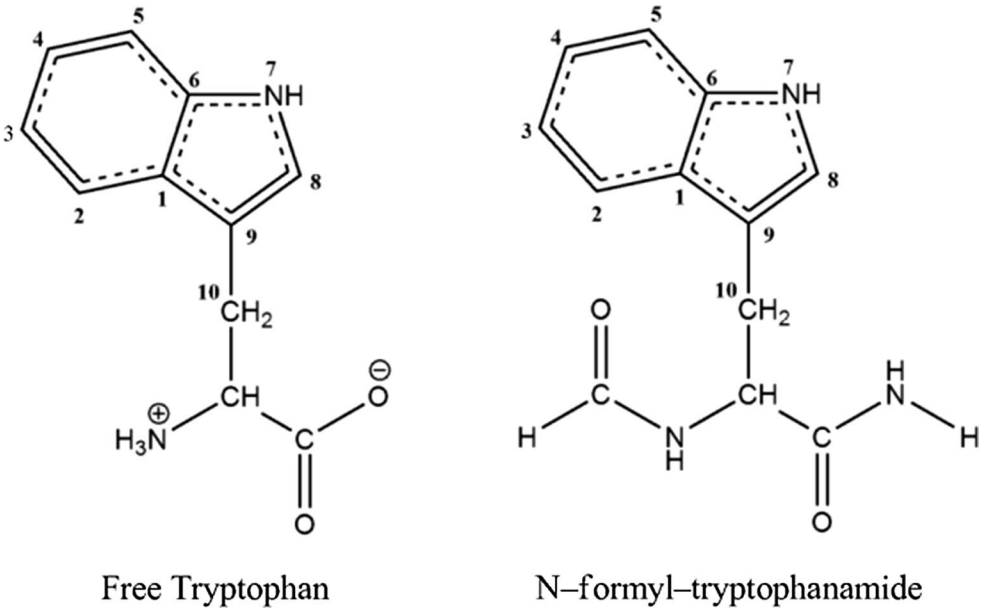
Tryptophan is a natural alpha amino acid that has several and important biological functions. For example, it is a precursor of neurotransmitters like melatonin and serotonin, and it is also an important building block for proteins, particularly enzymes [24]. Tryptophan also has been assumed to possess radical scavenging activity: it suppresses lipid peroxidation [25], and it is a good hydroxyl radical scavenger [26]. However, it is not well known whether it, itself, is responsible for this activity, or whether it is because of its metabolites, mainly hydroxylated metabolites [26, 27]. It also has been reported that tryptophan metabolites exhibit high scavenging ability toward reactive oxygen and chlorine species [28]. On the other hand, it has been recently proposed that tryptophan itself is not particularly sensitive to oxidative stress conditions. In fact, it was predicted to be rather inactive both as antioxidant and as a molecular target when attacked by hydroperoxyl radicals ($^{\cdot}$OOH), albeit it rapidly reacts with high reactive free radicals, such as $^{\cdot}$OH [26].

The main purpose of this work is to provide thermochemical and kinetic data on the mechanisms of the reactions between $NO_2^{\cdot}$, $NO^{\cdot}$ and $CO_3^{\cdot -}$ radicals and both free and peptide tryptophan models (Fig. 1).

Focusing on *N*-formyl-tryptophanamide (see Fig. 1) as a model for tryptophan as a protein residue, the same or similar small models for other amino acids have been previously used to study protein damage, please see Refs. [29, 30] and references therein.

Even though this type of model has been widely used and accepted, we would like to emphasize why it is adequate. At first, it might seem too simple to model something that occurs in a protein. What makes proteins unique is their tertiary and quaternary structure, which determines their 3D shape and is essential for performing certain functions. In order to mimic such processes (e.g., protein folding, enzymatic reactions), the theoretical methods applied (e.g., hybrid methods such as QM/MM) must include a very large system in order to account for all important interactions. On the other hand, proteins are not designed to be oxidized. Oxidation is an undesirable process that can involve one residue only, located in any protein region. Since usual oxidants are not protein targets, no specific protein orientation or conformation is necessary for the oxidation to take place. Moreover, it is well known that oxidative attacks occur randomly and non-specifically because of the very high

Fig. 1 Tryptophan models



reactivity and low selectivity of the oxidants in many cases. In addition, because of the lack of unsaturation in protein backbones, electronic effects cannot propagate further than two sigma bonds. Consequently, the rest of the protein has no important effect on the oxidation process, and a simplified model like the one used in this study is adequate to study protein damage and repair. Furthermore, this molecular model of proteins has also been tested experimentally [20]. The measured rate constant for the reaction of the *N*-ac-TrpH-NH_2 radical with ascorbate is 1.40×10^8 M^{-1} s^{-1} [20] (Table 1), while the repair of the same lateral amino acid damaged residue (i.e., tryptophanyl radical) in chymotrypsin, pepsin, lysozyme and β-lactoglobulin with ascorbate led to *k* values of 1.60×10^8 M^{-1} s^{-1}, 1.80×10^8 M^{-1} s^{-1}, 8.30×10^7 M^{-1} s^{-1} and 2.20×10^7 M^{-1} s^{-1} [20], respectively. Of the four proteins studied, the greatest discrepancy in *k* for the amino acid repair between a protein and the model is found with β-lactoglobulin, relative to which the *k* of the model is only six times larger. However, the agreement in *k* values with the other three proteins is very good. Therefore, the validity of the simplified molecular model for proteins used in this paper has been demonstrated. Additional examples of publications where very similar models have been used are listed in References [19, 31–39].

2 Computational details

Electronic calculations were performed within the framework of the density functional theory [40, 41] (DFT). The geometry optimizations and frequency calculations have been carried out in aqueous environment with the solvation model based on density (SMD) [42] using the M05-2X functional [43] and the 6-31+G(d,p) basis set. The M05-2X functional has been recommended for kinetic calculations by their developers, and it has been also successfully used by independent authors to that purpose [44–48]. SMD is considered to be a universal solvation model, due to its applicability to any charged or uncharged solute in any solvent or liquid medium for which a few key descriptors are known [42].

Unrestricted calculations were used for open shell systems and local minima and transition states were identified by the number of imaginary frequencies (NIMAG = 0 or 1, respectively). In the case of the transition states, it was verified that the imaginary frequency corresponds to the expected motion along the reaction coordinate, by intrinsic coordinate calculations (IRCs). All the electronic calculations were performed with Gaussian 09 software [49]. Thermodynamic corrections at 298.15 K were included in the calculation of relative energies. In addition, the solvent cage effects have been included according to the corrections proposed by Okuno [50], taking into account the free volume theory of Benson [51].

The rate constants (*k*) were calculated using the conventional transition state theory (TST) [52–54] and 1 M standard state, following the quantum mechanics-based test for overall free radical scavenging activity (QM-ORSA) protocol [55]. This computational protocol has been validated by comparison with experimental results, and its uncertainties have been proven to be no larger than those arising from experiments [55].

In the case of rate constants limited by diffusion, the Collins–Kimball [56] theory was employed. This is used

in combination with the Smoluchowski [57] approximation for calculation of diffusion constants for an irreversible bimolecular diffusion-controlled reaction, and with Stokes–Einstein theory [58, 59] to calculate the diffusion coefficients of the reacting species.

3 Results and discussion

As commented before, two different models of alpha amino acid tryptophan have been studied. They are *N*-formyl-tryptophanamide as a model of tryptophan residues in proteins (PT) and free zwitterionic tryptophan (ZT), previously used for such purpose (Fig. 1) [26]. The reaction Gibbs free energies ($\Delta G°$, kcal/mol) were calculated as:

$$\Delta G^{\circ}_{\text{reaction}} = \sum G^{\circ}_{\text{products}} - \sum G^{\circ}_{\text{reactants}}, \quad (1)$$

their values are shown in Tables 2 and 3 for the ZT and PT models, respectively, in aqueous solution at physiological pH. For that, different reaction mechanisms have been considered (Scheme 1), those are:

Single electron transfer (SET):

$$ZT + R^{\cdot} \rightarrow ZT^{+\cdot} + R^{-}$$

$$ZP + R^{\cdot} \rightarrow ZP^{+\cdot} + R^{-}$$

Radical adduct formation (RAF):

$$ZT + R^{\cdot} \rightarrow [ZT - R]^{\cdot}$$

$$ZP + R^{\cdot} \rightarrow [ZP - R]^{\cdot}$$

Hydrogen transfer (HT):

$$ZT + R^{\cdot} \rightarrow ZT^{\cdot}_{-H} + HR$$

$$ZP + R^{\cdot} \rightarrow ZP^{\cdot}_{-H} + HR$$

Table 2 Gibbs free energies of reaction ($\Delta G°$, kcal/mol), for free zwitterionic tryptophan (ZT) with the $NO_2^{\cdot}$, $NO^{\cdot}$ and $CO_3^{\cdot -}$ radicals in aqueous solution, at 298.15 K

	$NO_2^{\cdot}$	$NO^{\cdot}$	$CO_3^{\cdot -}$
SET	2.39	79.64	4.21
HT			
n7	5.93	75.93	−15.09
c10	1.87	71.86	−19.16
RAF			
c2	26.48	31.16	−5.19
c3	14.06	–	2.45
c4	11.28	–	1.22
c5	9.69	–	−2.84
c8	4.90	–	−12.70

Table 3 Gibbs free energies of reaction ($\Delta G°$, kcal/mol), for protein residue tryptophan (PT) model with the $NO_2^{\cdot}$, $NO^{\cdot}$ and $CO_3^{\cdot -}$ radicals in aqueous solution, at 298.15 K

	$NO_2^{\cdot}$	$NO^{\cdot}$	$CO_3^{\cdot -}$
SET	−0.40	76.84	1.41
HT			
n7	3.81	104.74	−16.80
c10	2.57	103.49	−18.05
RAF			
c2	24.91	–	−4.87
c3	23.91	–	3.05
c4	24.16	–	0.61
c5	8.29	–	0.43
c8	2.26	–	−10.99

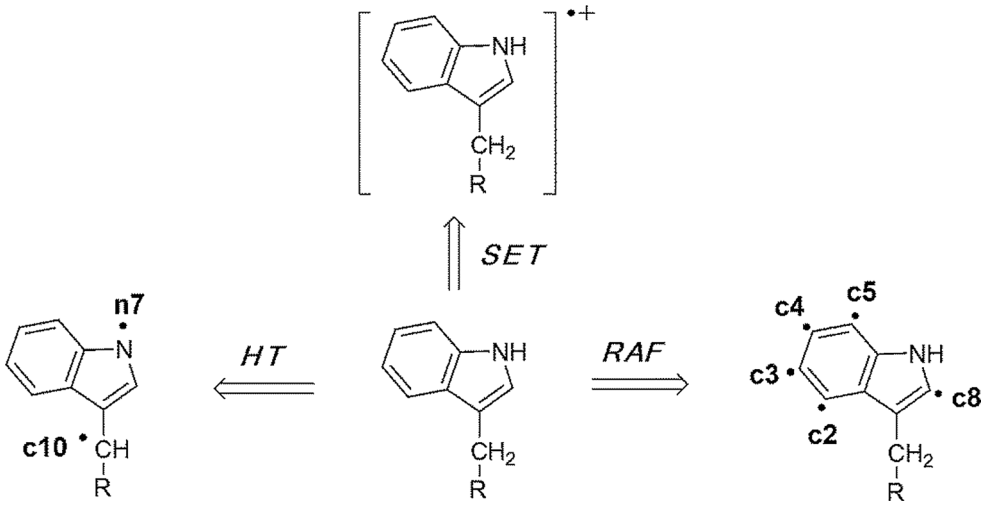
The first and very important conclusion obtained from the thermodynamic results of Tables 2 and 3 is that $NO^{\cdot}$ does not react with tryptophan in any way. The reaction Gibbs free energies for all possible reaction channels are very endergonic. In addition, radical adduct formation is not viable with any of the neutral tryptophan species (ZT and PT models), i.e., the optimization of these adducts gives dissociated products and their formation is not possible. Therefore, the Gibbs free energies of reaction for ZT in sites c3, c4, c5 and c8, and for PT in sites c2, c3, c4, c5 and c8 are not reported. These results are correct beyond any possible inaccuracies in the calculations, and they wouldn't be modified by changing to more reactive targets than tryptophan. Therefore, we can safely conclude that $NO^{\cdot}$ is not an RNS by itself.

The $CO_3^{\cdot -}$ radical is the most reactive of the three studied radicals toward both tryptophan species. It yields the most exergonic values, the thermodynamically favorable sites being n7 and c10 for the formal hydrogen transfer (HT) mechanism in both models, with values ranging from −15.00 to −19.00 kcal/mol. For the radical adduct formation (RAF) mechanism, addition occurs at c2 and c8 in PT and at c2, c5 and c8 in ZT, with values ranging from −5.00 to −11.00 kcal/mol, and from −3.00 to −13.00 kcal/mol, respectively. The variations of Gibbs free energy for the single electron transfer (SET) mechanism are slightly endergonic.

The data reported in Tables 2 and 3 show that the reaction Gibbs free energies of the $NO_2^{\cdot}$ radical for HT reactions are endergonic for all positions. For the SET mechanism, the values are close to 0 kcal/mol, with only one slightly exergonic value for the tryptophan PT model (−0.40 kcal/mol).

It is important to take into account that the SET mechanism produces a tryptophan radical cation that is very acid and immediately deprotonates, giving a more stable tryptophanyl radical. For this reason, slightly endergonic reactions could still play an important role in the overall reaction. From thermodynamic data, it seems that both radicals

Scheme 1 Reaction channels of SET, HT and RAF mechanisms



have similar reactivity toward tryptophan residues via SET. As will be shown latter, the hypothesis that SET is not important for $CO_3^{\cdot-}$ and $NO_2^{\cdot}$ and therefore that the latter is not reactive at all would be incorrect.

It seems important to note that while both radicals are similarly capable of removing an electron from tryptophan models, they dramatically differ in the capability of abstracting an H atom or adding to tryptophan. Thus, $CO_3^{\cdot-}$ is apparently reactive, or at least thermodynamically allowed to react, via SET, HT, and RAF, while $NO_2^{\cdot}$ seems to be able to react only via SET.

Based only on thermodynamic data, we can conclude that $NO^{\cdot}$ does not react with tryptophan in any way and also that $NO_2^{\cdot}$ cannot react via HT or RAF mechanisms. We can also expect that $NO_2^{\cdot}$ reacts via a SET mechanism and that $CO_3^{\cdot-}$ reacts via any mechanism. However, without considering kinetics it is not possible to assess the absolute or relative importance of these reactions.

Accordingly, to provide further insight on the scavenging activity of ZT and PT and on the origin of the damage induced by $NO_2^{\cdot}$, $NO^{\cdot}$ and $CO_3^{\cdot-}$ radicals, we have performed a kinetic study on all the reaction channels that

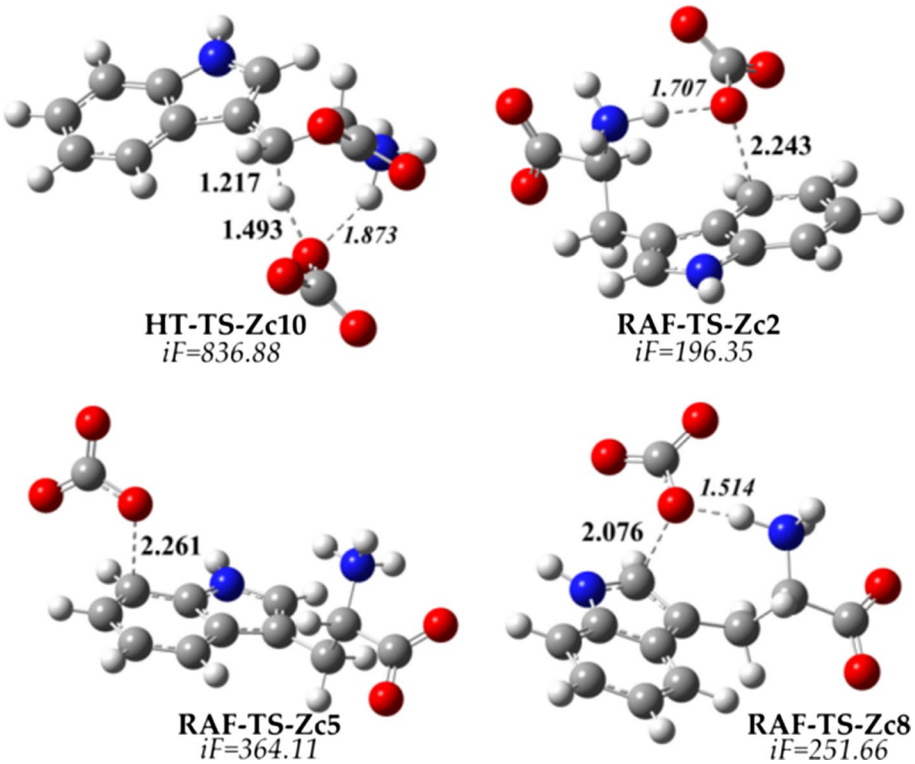


Fig. 2 Transition states of free tryptophan (ZT) with $CO_3^{\cdot-}$ radical in aqueous solution, at 298.15 K

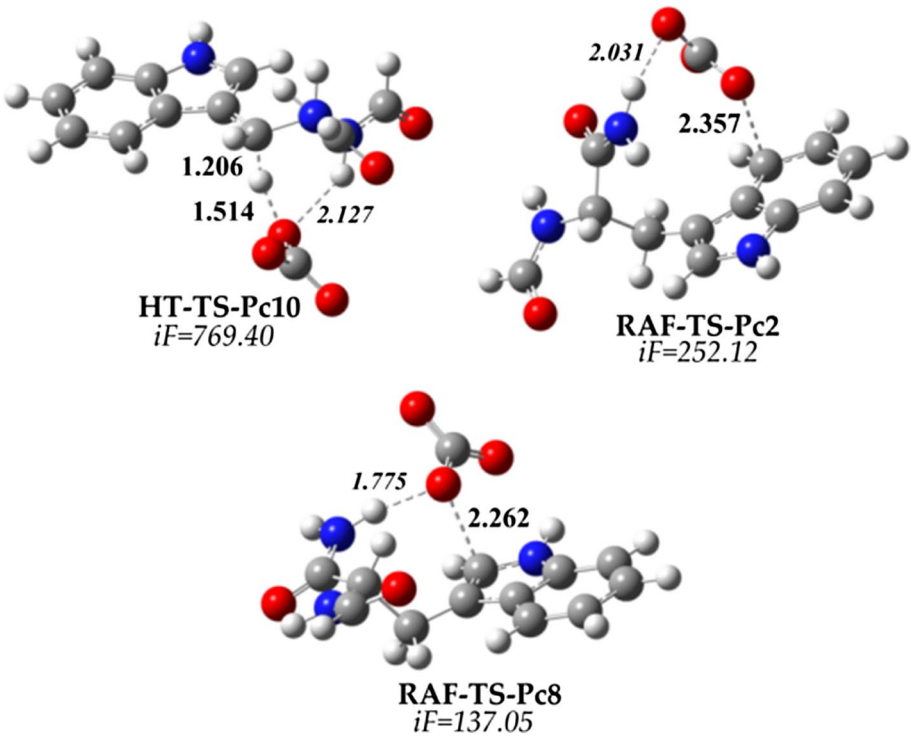


Fig. 3 Transition states of protein residue tryptophan model (PT) with $CO_3^{\cdot-}$ radical in aqueous solution, at 298.15 K

are not forbidden by thermodynamic results. We have calculated the transition states of $CO_3^{\cdot-}$ with the two tryptophan models (Figs. 2, 3) only for the thermochemically favored sites in the HT and RAF mechanisms. We have also obtained the Gibbs free energies of activation for the SET mechanism with $CO_3^{\cdot-}$ and $NO_2^{\cdot}$ since they might be important, provided that their endergonic values are not too large. In this case, they might represent significant reaction pathways because they can take place at significant rates constants and evolve to more stable products via acid–base equilibrium.

Table 4 contains the Gibbs free energies of activation (kcal/mol) for both models of tryptophan. The calculated barriers for the SET mechanism from $NO_2^{\cdot}$ to both models of tryptophan are very similar, with a difference of only 0.06 kcal/mol. The barrier values are 8.54 and 8.60 kcal/mol for ZT and PT, respectively. Regarding the $CO_3^{\cdot-}$ SET mechanism, the reaction barriers are 5.11 and 4.81 kcal/mol for the ZT and PT models, respectively. Concerning RAF reactions, ZT has smaller barriers with respect to the other model of tryptophan. Site c8 has the smallest Gibbs free energies of activation in both cases: in free tryptophan, this value is ~0 kcal/mol. The only reason for such an atypical low barrier is that the transition state is stabilized by a very strong H bond, with an interaction distance of 1.51 Å between the attacking radical and the NH_3 of the zwitterion (Fig. 2). On the contrary, for the protein residue model, the positively charged NH_3 moiety does not exist and the H bond with the amidic H is weaker (Fig. 3). In the case of the HT mechanism, the barriers for site c10 are 12.77 and 14.74 kcal/mol for ZT and PT models, respectively. For HT from the n7 site, no transition state is reported because the Gibbs free energy of activation can be considered equal to zero and the reaction is limited by diffusion.

At this point, it seems worthwhile to call attention to the fact that there are some inconsistencies in the literature regarding the *p*Ka of $HCO_3^{\cdot}$. It has been reported to be equal to 7.6 [60], which means that 38.7 % of the carbonate is in the form of $CO_3^{\cdot-}$ and 61.3 % exists as $HCO_3^{\cdot}$ at physiological pH. However, according to Ref. [61] "A pulse radiolysis study which uses a flow system to irradiate mixtures of H_2CO_3 and HCO_3^- within 50 ms of their formation has demonstrated that the carbonate radical is a strong acid, *p*Ka < 0, contrary to published reports of high *p*Ka's." We have also calculated the *p*Ka of $HCO_3^{\cdot}$ and obtained a value of −6.4, i.e., $HCO_3^{\cdot}$ is a very strong acid. This supports the findings from Ref. [61] and validates the reliability of the calculations presented here, SMD/M05-2X/6-31+G(d,p), in particular solvation energies. Therefore, $CO_3^{\cdot-}$ is the only acid–base species relevant to our investigation, and measured rate constants should be pH independent.

To obtain reactivity values that can be directly compared with experimental data, we have calculated the rate constants corresponding to the activation barriers reported in Table 4. The rate constants are tabulated in Table 5. It can be observed that the rate constant for the $NO_2^{\cdot}$ reaction with the ZT model of tryptophan is 3.43×10^6 M^{-1} s^{-1}. This rate constant is in good agreement with reported values:

Table 4 Gibbs free energies of activation ($\Delta G^{\neq}$, kcal/mol), for free (ZT) and protein residue tryptophan (PT) models with the $NO_2^{\cdot}$ and $CO_3^{\cdot-}$ radicals in aqueous solution, at 298.15 K

	ZT $NO_2^{\cdot}$	PT	ZT $CO_3^{\cdot-}$	PT
SET	8.54	8.60	5.11	4.82
HT				
n7	–	–	0.00	0.00
c10	–	–	12.77	14.74
RAF				
c2	–	–	7.20	11.33
c3	–	–	–	–
c4	–	–	–	–
c5	–	–	11.04	–
c8	–	–	0.00	5.19

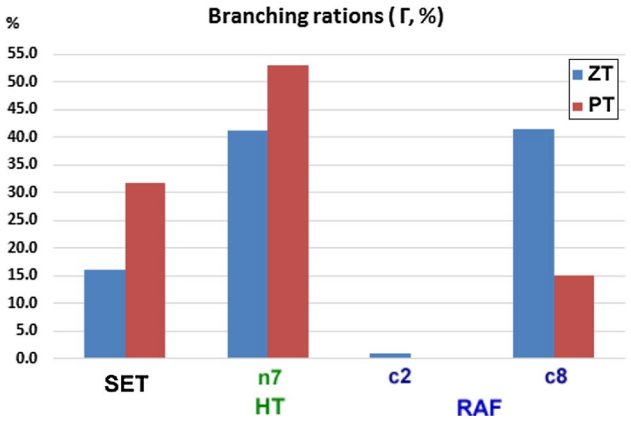


Fig. 4 Branching ratios (Γ, %) for free (ZT) and protein residue tryptophan (PT) models with $CO_3^{\cdot-}$ radicals in aqueous solution, at 298.15 K

it is only 3.1 higher than the experimental rate constant reported by several authors (~1.00–1.10 × 10^6 M^{-1} s^{-1}) [9, 19, 21]. The corresponding calculated rate constant with the protein residue model is 3.09 × 10^6 M^{-1} s^{-1}.

Several experimental values have been reported at different pHs for the rate constant of the tryptophan reaction with $CO_3^{\cdot-}$. However, this reaction should be pH independent, and it is not clear whether any corrections have been done using incorrect *p*Ka values. The experimental values range from 1.00 × 10^8 M^{-1} s^{-1} to 7.00 × 10^8 M^{-1} s^{-1} [9, 22], i.e., the deviations are small but not negligible. Our calculated overall rate constants are equal to 5.98 × 10^9 M^{-1} s^{-1} and 4.65 × 10^9 M^{-1} s^{-1} for free and protein models. These results are in agreement with the experimental data, albeit slightly overestimated. The calculated value is overestimated about 8.5 times, while one experimental value is 7 times higher than the other. These results validate the mechanistic study provided here as well as the described reactivity of the protein residue model.

According to the data in Table 5, the $NO_2^{\cdot}$ radical reacts exclusively via SET, with rate constants of 3.43 × 10^6 M^{-1} s^{-1} and 3.09 × 10^6 M^{-1} s^{-1} for ZT and PT models, respectively. These values are two orders of magnitude smaller than the corresponding rate constants for the $CO_3^{\cdot-}$ radical, again in agreement with experimental data.

Figure 4 shows the branching ratios for the main reactions between both tryptophan models and the $CO_3^{\cdot-}$ radical. Reaction paths c5 and c10 were found to be negligible and c2 almost negligible, regardless of the used model. For ZT, the contributions of HT from site n7 and RAF at site c8 are almost identical. The next highest contribution corresponds to the SET mechanism. For the PT model, HT from site n7 represents, by far, the largest contribution, followed by SET. The RAF mechanism at site c8 contributes about 15 %. It is important to mention that the $CO_3^{\cdot-}$ radical is reactive through the three investigated mechanisms. In that sense, it is similar to the $^{\cdot}OH$ radical although, being less reactive, it could be more selective. This implies that in a

Table 5 Rate coefficients (k_{app}, M^{-1} s^{-1}) and branching ratios (Γ, %) for free (ZT) and protein residue tryptophan (PT) models with the $NO_2^{\cdot}$ and $CO_3^{\cdot-}$ radicals in aqueous solution, at pH = 7.4 and 298.15 K

	ZT k_{app} ($NO_2^{\cdot}$)	PT	ZT k_{app} ($CO_3^{\cdot-}$)	PT	ZT Γ ($CO_3^{\cdot-}$)	PT
SET	3.43E+06	3.09E+06	9.69E+08	1.48E+09	16.22	31.92
HT						
n7	–	–	~2.47E+09	~2.47E+09	~41.33	~53.04
c10	–	–	5.41E+03	1.74E+05	0.0001	0.004
RAF						
c2	–	–	6.35E+07	3.10E+04	1.06	0.001
c3	–	–	–	–	–	–
c4	–	–	–	–	–	–
c5	–	–	1.01E+05	–	0.002	–
c8	–	–	2.47E+09	6.99E+08	41.39	15.04
$k_{overall}$	3.43E+06	3.09E+06	5.98E+09	4.65E+09		

protein it may react mainly with tryptophan, tyrosine and cysteine.

From the above kinetic data, we suggest that free tryptophan is a good scavenger of $NO_2^{\cdot}$ and $CO_3^{\cdot-}$ radicals, especially of the latter, and that it can inhibit the damage induced by these radicals to biological targets. However, since the rate constants of both models are very similar, proteins containing tryptophan could be damaged by $NO_2^{\cdot}$ and $CO_3^{\cdot-}$.

4 Conclusions

Density functional theory was used to determine and quantify the overall rate coefficients and thermochemical data for the reactions between $NO_2^{\cdot}$, $NO^{\cdot}$ and $CO_3^{\cdot-}$ radicals and two tryptophan models: free zwitterionic tryptophan and a model for tryptophan in proteins.

According to the thermochemical data, and taking into account that all possible mechanisms were explored, we conclude that the $NO^{\cdot}$ radical is not capable of reacting with tryptophan. Moreover, since tryptophan is one of the most reactive amino acids, and our results show that the $NO^{\cdot}$ radical is an extremely poor acceptor of both H atoms and electrons, it is highly improbable that $NO^{\cdot}$ itself could react with any amino acid or even any antioxidant via its oxidation. In other words, it is safe to conclude that it is not a nitrogen reactive species. Any reaction attributed to $NO^{\cdot}$ should be rationalized in terms of $NO^{\cdot}$ as a precursor of other species. The latter could be the products of its primary reaction with, for example, O_2 or $O_2^{\cdot-}$.

The $NO_2^{\cdot}$ radical reacts only via SET, with large, albeit not, diffusion-controlled rate constants. $NO_2^{\cdot}$ is not a good H atom acceptor, neither does it add to double bonds. It could be a testing radical for molecules which are presumed to react via this mechanism. For example, it would not react with glutathione at acid pH, but it will react fast with it at basic pH. Free tryptophan can be considered a reasonable scavenger of $NO_2^{\cdot}$ radicals via a SET mechanism.

$CO_3^{\cdot-}$ reacts with rate constants that are close to diffusion limit, regardless of the assumed mechanism. It reacts very fast by abstracting an H-N hydrogen atom via a formal H transfer mechanism. It reacts also very fast via SET and also via a RAF at the c8 carbon atom. Thus, tryptophan can be considered to be an excellent $CO_3^{\cdot-}$ scavenger. The HT reaction could be important, because it means that $CO_3^{\cdot-}$ could damage aliphatic amino acids, since they react only via HT; therefore, they are expected to be inert to $NO_2^{\cdot}$ $CO_3^{\cdot-}$ is more reactive than $NO_2^{\cdot}$ via SET, a fact that could not be anticipated from thermodynamic calculations.

The agreement of our results with experimental values validates these conclusions, including the mechanistic study, the inertness of $NO^{\cdot}$, as well as the reactivity of our protein residue model.

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